REMARKS

Claims 1-17 were pending in the present application. By this Amendment, Applicants have removed the word "such" from claims 1, 9, and 11 to address the Examiner's concerns set forth in the Office Action. Support for the amendments to the claims can be found in the specification and claims as originally filed. The amendments to the claims introduce no new matter. Upon entry of the present Amendment, claims 1-17, as amended, will be pending and under examination.

The January 15, 2004 Office Action

Rejections under 35 U.S.C. § 112, first paragraph – *Written Description*

The Examiner rejected claims 1-8 under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirement. In particular, the Examiner alleged that there is no support in the specification for the term "reducing invasiveness and/or migration of malignant cells."

In response, Applicants respectfully traverse the Examiner's rejection. Definitions for the terms "invasiveness" and "migration" are set forth at page 3, lines 8-17 in the specification. In particular, "invasiveness" is defined as the "ability of cells to cross anatomic barriers, such as basal membranes, interstitial stroma and intracellular junctions which divide tissue compartments." "Migration" is defined as "one of the steps of invasion, motility, which allows tumour cells to cross basal membrane and stroma."

The experimental section of the specification, beginning at page 4, describes tests using the artificial basal membrane MATRIGEL, which allows only the crossing of malignant cells, but not

other cell types (page 4, line 33 to page 5, line 4). Using this assay, it could be determined that, compared to untreated malignant cells, treatment of malignant cells with 0.1-1μM lipoic acid led to a significant inhibition of the invasiveness of those malignant cells (tables 1-3).

It is clear from the above, and the disclosure as a whole, that Applicants were in possession of the claimed invention at the time the application was filed and that the clause that forms the basis of the Examiner's rejection is sufficiently supported in the specification as filed. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the written description rejection of claims 1-8.

Rejections under 35 U.S.C. § 112, first paragraph – *Enablement*

The Examiner rejected claims 1-17 under 35 U.S.C. §112, first paragraph, as not being fully enabled by the specification. In particular, the Examiner acknowledged that the specification enables the specific cancers disclosed on page 25 [sic], lines 2-3, but asserted that it does not provide enablement for all cancers. Applicants note that the specification does not contain a page 25. Seeking clarification, Applicants' attorney had telephoned the Examiner, who took the position that only the treatment of human fibrosarcoma and of HT1080 cells are enabled (page 9, lines 2 and 3).

Applicants respectfully traverse the Examiner's rejection. Applicants' invention is directed to, *inter alia*, a method for reducing invasiveness and/or migration of malignant cells in a patient in need of a reduction in the invasiveness and/or migration of malignant cells comprising: administering to the patient an active ingredient consisting essentially of at least one alpha lipoic acid

or at least one physiologically equivalent derivative thereof in an invasiveness and/or migration of malignant cells reducing effective amount, and a pharmaceutically acceptable carrier therefor. There is an established correlation between the metastatic behavior of malignant cells in general and the crossing of a basal membrane by such malignant cells. Applicants refer the Examiner to the Albini et al. reference cited on page 5, lines 5-7 of the specification as evidence of the state of the art at the time the application was filed, and as evidence of the recognition in the art of this correlation. Applicants emphasize that the findings of Albini, et al. are widely accepted in the art, as documented by the frequent citation of this article in the relevant literature. See, e.g., http://cancerres.aacrjournals.org/cgi/content/abstract/47/12/3239. A copy of the Albini, et al. reference is attached for the Examiner's convenience.

Additional Experimental Results

Applicants submit the following additional experimental results which further support the full enablement of the claims:

Invasiveness is characteristic of all metastatic cells, irrespective of their origin. LA was shown to inhibit the motility and invasiveness of MDA-MB-231 cells (deriving from a human metastatic breast carcinoma) and HuMi Ttu-2 cells (tumorigenic cell line selected from human milk cells immortalized with SV40), besides the HT1080 cells (human fibrosarcoma cells) disclosed in the patent application. Furthermore, LA was shown to significantly inhibit the anchorage independent growth - which is indicative of malignant transformation - of HuMi Ttu-2 and MCF-7 cells (metastatic human breast carcinoma).

Table 1. Effects of LA treatment on the chemotactic migration of human breast cancer cells

	MDA-MB-231		TTu2	
LA (μM)	No. cells ± S.E.	% inhibition	No. cells ± S.E.	% inhibition
0	252 ± 7		36 ± 2	
100	214 ± 29	15	35 ± 3	3
500	171 ± 25	32	19 ± 1	49
1000	115 ± 12	54	ND	ND

Table 2. Effects of LA treatment on the invasive behavior of human breast cancer cells

	MDA-MB-231		TTu2	
LA (μM)	No. cells ± S.E.	% inhibition	No. cells ± S.E.	% inhibition
0	173 ± 19		37 ± 3	
100	172 ± 18	1	34 ± 3	7
500	83 ± 4	52	23 ± 1	37
1000	56 ± 21	68	ND	ND

Table 3. Effects of LA treatment on the anchorage independent growth of human breast cancer cells

	MCF7		TTu2	
LA (μM)	No. colonies ± S.E.	% inhibition	No. colonies ± S.E.	% inhibition
0	708 ± 4		294 ± 3	
10	420 ± 10	41	84 ± 3	71
100	313 ± 6	56	25 ± 13	92
500	125 ± 8	82	13 ± 1	96
1000	67 ± 3	90	ND	ND

Breast tissues are a main target of LA. The data *in vitro* have been confirmed in a preliminary study where LA was particularly effective in modulating proliferation markers in breast neoplastic lesions. LA derivatives, such as DHLA (dihydrolipoic acid) and LA amide (tioctamide, TOA) showed an activity similar to LA.

Table 4. Effects of LA derivatives on the chemotactic migration of 1,2-DBE transformed cells

	LA		DHLA		TOA	
(μM)	No. cells ± S.E.	% inhibition	No. cells ± S.E.	% inhibition	No. cells ± S.E.	% inhibition
0	183 ± 6		183 ± 6		183 ± 6	
100	114 ± 8	38	132 ± 5	28	147 ± 1	20
500	116 ± 26	37	131 ± 3	28	115 ± 3	37

Table 5. Effects of LA derivatives on the invasive behavior of 1,2-DBE transformed cells

	LA		DHLA		TOA	
(μM)	No. cells ± S.E.	% inhibition	No. cells ± S.E.	% inhibition	No. cells ± S.E.	% inhibition
0	94 ± 4		94 ± 4		94 ± 4	
100	90 ± 3	4	50 ± 5	47	61 ± 3	35
500	58 ± 2	38	43 ± 3	54	43 ± 5	55

The LA activity on tumor cells was further investigated with the cDNA microarray technique, in order to find intracellular effectors of LA. This technique allows one to analyze differences in the expression levels of thousands of genes, by comparing polyA+ RNAs from two cell populations - in this case, cells treated with 100 μM LA, and untreated cells.

First, LA-induced gene modulation was analyzed in DBE/F4 cells (100 μM, 6 hrs). Genes responsible for LA transformation/elimination (tyreodoxin, reductase, phosphogluconolacton dehydrogenase decarboxylase, GSH-T) were overexpressed in LA-treated cells. Afterwards, the effect of LA (100 μM, 24 hrs) on HepG2 cell line (human hepatocarcinoma) was investigated. The results of cDNA microarray assays reveal that:

- LA modulates many genes;
- Genes involved in metabolic processes (glycolysis, Krebs cycle, cholesterol biosynthesis, fatty acid degradation) are overexpressed;
- Genes involved in signal-transduction pathways, in cell-cell interaction and in matrix adhesion are down-regulated.

These observed effects are either indicative of LA-mediated pathways, or are the result of a signal-transmission control mechanism through negative feed-back.

In addition to these experimental results, and the Albini reference noted above, Applicants further provide, attached hereto, four review articles describing anti-metastatic therapy and its differences from chemotherapy. These include:

1. Timar, J, et al., Molecular Pathology of Tumor Metastasis III, (2003), Pathology Oncology Research, Vol. 9, No. 1.

2. Sava, G., et al., Drug Control of Solid Tumour Metastases: A Critical View, (1999), Anticancer Research 19:1117-1124.

3. Gupta, M..K., et al., Mechanism and its Regulation of tumor-induced angiogenesis (2003), World J. Gastroenterol. 9(6):1144-1155.

4. Chambers, A.F., et al., Clinical targets for Anti-Metastasis Therapy, Advances in Cancer Research, Vol. 79,:91-121.

Finally, the specification itself includes various statements in support of the correlation between the metastatic behavior of malignant cells in general and the crossing of a basal membrane by such malignant cells. For example, the specification notes that "the number of metastatic cells crossing the MATRIGEL and their malignant behavior are directly related" (Page 5, lines 5-7) and "the results clearly indicate the anti-invasive dose-related effect of lipoic acid" (Page 9, lines 26-27, discussing the results of the chemoinvasion assay). Applicants note that the Examiner has not set forth any reason to doubt the truth or accuracy of the statements made in the specification, as

required by applicable case law. In re Marzocchi, 169 USPQ 367, 370 (CCPA 1971).

In light of the above, Applicants assert that the claims as written are fully enabled by the specification. Applicants therefore respectfully request that the Examiner reconsider and withdraw the enablement rejection of claims 1-17.

Examiner's rejection under 35 U.S.C. § 112, second paragraph – *Indefiniteness*

The Examiner rejected claims 1-17 under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. In particular, the Examiner asserted that, in claim 9, the nature of the adhesion of the malignant cells is not clear. The Examiner suggested that limiting the claim to "adhesion to a basal membrane", as recited in claim 10.

In response, Applicants respectfully traverse this ground of the Examiner's indefiniteness rejection. Applicants refer the Examiner to the definition of the term "adhesion" on page 3, lines 22-23 of the specification, which notes that "adhesion" refers to the ability of the cells to to specifically recognize and attach to extra-cellular matrix. In view of this definition, one of ordinary skill in the art would readily understand the meaning and ascertain the scope of the term "adhesion to a basal membrane" as it is used in the claims.

The Examiner also rejected claims 1, 9, and 11, under 35 U.S.C. §112, second paragraph, as being indefinite in their recitation of the word "such." According to the Examiner, this term renders the claims indefinite because it is unclear whether the limitations following the word are part

of the claimed invention.

Applicants disagree with the Examiner's position. Nevertheless, without conceding the correctness of the Examiner's position, but to expedite allowance of the subject application, Applicants have amended claims 1, 9, and 11 to further clarify these claims by removing the term "such." Applicants believe that these clarifying amendments fully address and overcome the Examiner's concerns. In light of the Amendments and remarks in response to the Examiner's rejection under 35 U.S.C. § 112, second paragraph, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 1, 9, and 11.

Examiner's rejection under 35 U.S.C. § 103

The Examiner rejected claims 1-17 under 35 U.S.C. §103(a) as obvious in view of International Appl. No. WO99/06040 (Berry, et al.) According to the Examiner, Berry, et al. discloses using alpha lipoic acid or derivatives thereof orally or parenterally to treat cancer metastasis. The Examiner acknowledged that the instant claims differ from the Berry reference in reciting that the active ingredient consists essentially of only an alpha lipoic acid or physiologically equivalent thereof, whereas in Berry, et al., both a tocotrienol and alpha lipoic acid are used. According to the Examiner, however, Berry, et al. discloses that each compound is effective and that the purpose of using them is for synergistic effect. It is the Examiner's position that there is ample motivation to use the lipoic acid alone, because Berry, et al. discloses that it is effective to treat cancer metastasis. The Examiner stated, for example, that the lipoic acid can be used alone to

measure baseline response to treatment or to determine if the tocotrienol causes an adverse effect.

In response, Applicants respectfully traverse the Examiner's rejection. Berry, et al. refers to tocotrienol's and alpha lipoic acid's ability in "reducing or blocking inflammatory responses and [in] regulating the activity of transcription factors sensitive to a cell redox state, such as the DNA transcription factor NF-kappa B." (page 13, lines 12-16).

From this and the description as a whole, it is apparent that the focus of Berry is on diseases in which free radicals have been found to be a causative factor. A cancer that is described to be at least partially caused by such free radicals is lung cancer (page 17, last paragraph).

The Examiner suggests that if an adverse effect to tocotrienol was observed, one of skill in the art would equally resort to any strong antioxidant, of which there are many, to seek to achieve the desired result, i.e., free radical suppression to treat one of the many diseases listed on page 15. Applicants assert, however, that there is simply no motivation in the reference to select, among the many antioxidants available, the one that is disclosed in the present application, to have primary usefulness when used together with tocotrienol. Furthermore, the disclosure of cancer metastasis on page 15 as a disease that is "believed to be caused at least in part" by the effect of reactive oxygen species, does not provide the person skilled in the art with a reasonable expectation of being successful in reducing cancer metastasis with tocotrienol and alpha lipoic acid, and certainly not with alpha lipoic acid alone. Berry, et al., therefore, does not render Applicants' claims obvious. Accordingly, Applicants respectfully request that the Examiner reconsider and withdraw the rejection of claims 1-17 under 35 U.S.C. §103(a).

In view of the above remarks, amendments, and evidence, Applicants believe that the Examiner's rejections set forth in the January 15, 2004 Office Action have been fully overcome and that the present application is in condition for allowance. The Examiner is invited to telephone the undersigned if it is deemed to expedite allowance of the application.

Respectfully submitted,

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Attachments: Albini, et al. article
Four review articles

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